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THE ANATOMY OF THE ACRIDIDÆAN HEART AND ITS HISTOLOGICAL STRUCTURE, ... *Lalia V. Walling.*

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THE ANATOMY OF THE *ACRIDIDÆAN* HEART AND ITS HISTOLOGICAL STRUCTURE.

BY LALIA V. WALLING.

(Contribution from the Zoölogical Laboratory, No. 184.)

Submitted in partial fulfilment of the requirements for the degree of Master of Arts.

Plate XXXVII.

CERTAIN interesting physiological experiments on the heart and respiratory activities in the grasshopper,[1] led to the investigations described in this paper. So many of the experimental results obtained resembled those described by Carlson[2] for *Limulus,* where he proved beyond a doubt the neurogenic theory of heart contraction, that I hoped to show the same for the grasshopper.

On account of the small size of the heart and its delicate structure this has not been an easy task. Many difficulties have beset the work, and while I cannot say beyond a doubt that nerve- or ganglion-cells have as yet been found, it seems worth while to give some account of the methods employed and the results obtained.

This work was pursued under the direction of Dr. C. E. McClung, to whom I am indebted for much valuable assistance. I am also obliged to Doctor Hyde, at whose suggestion this work was undertaken, for many helpful suggestions.

I was able to find very little literature touching on the anatomy of the heart of any of the insects or other invertebrates, and very much less concerning its histological structure. Dogiel[3] described cells which he called ganglion-cells around

1. Walling, L. V., Jour. of Exp. Zoöl., vol. III, No. 4, p. 821.

2. Carlson, E. J., Amer. Jour. of Phys., vol. XII, No. 1, '04, p. 67.

3. Dogiel, Joh., Memoires de L'Academie St. Petersburg, VII series, tome XXIV, No. 10, p. 9.

the heart of the *Corethra larva.* In only a few cases did he find processes on the cells. He also found ganglion-cells in the auricle of the snail's heart.

Ransom,[4] however, thinks Dogiel is mistaken in calling these cells ganglion-cells. Instead he thinks they are "wandering connective tissue" cells, probably the same as the "plasma-cells" described by Brock.[5] He found cells similar to Dogiel's ganglion-cells in the auricle of *Aplysia* and in *Pterotrachea* in the connective tissue all over the body. In *Helix* he found the "plasma-cells" in various stages of division, but in no case did he find *what he could call true ganglion-cells,* or nerve-cells, in the substance of the rhythmically contractile organ, but he did find nerve-fibers. *Octopus* and a tunicate were also examined by him, but with the same results.

In *Aplysia,* Ransom described these cells as being roughly spherical with deeply staining nucleus and a clear, slightly staining cell substance. In *Pterotrachea* the cells are roundish, possess a not very distinct oval nucleus placed near the periphery of the cell, and often a nucleolus. There is no definite capsule. In *Helix* these cells were found in various stages of division. They somewhat resemble cells that I found in the grasshopper, which will be described later.

Ransom also states that other cells which might be taken for nerve-cells are nothing more than ordinary connective-tissue cells. He says: "In no molluscan or tunicate heart examined are ganglion-cells to be found." Foster[6] thinks they do not exist, and Darwin,[7] Ransom and Biederman all say that the snail's heart has no ganglion-cells, although Ransom says nerve-fibers do enter the heart. Dogiel believes them to exist in the auricle.

Among the works of more recent investigators concerning the activity of the heart-muscle of the invertebrates, Carlson's[8] is perhaps the most extensive. Aside from his observation, the majority substantiate the myogenic theory of contraction. Carlson's work on invertebrates has included a few experiments on insects, and a microscopic examination of the heart of tarantula, polyphemus moth, and the grasshopper,

4. Ransom, Jour. of Phys., vol. 5, p. 324, 1884.

5. Brock, Zeit. f. Wiss. Zoöl., Bd. 39, 1883.

6. Foster, Pflüger's Archiv., vol. V, p. 191.

7. Darwin, Jour. of Anat. a. Phys., vol. X, part III, 1876.

8. Carlson, A. J., Amer. Jour. of Phys., vol. XV, 1906, No. II, p. 130.

but in no case did he find nerves in the heart or any connecting the heart with the central nervous system.

McClendon[9] found a nerve-cord on the dorsal side of the heart of the scorpion, and Beyne,[10] in his research on the origin of heart action, nervous or muscular, says that he found no nerve-cells in the snail's heart.

METHODS.

The most serious difficulty in connection with this research was to obtain a method by which the heart-tissue could be removed from the hard chitin and sectioned without injuring the structures.

The first method employed was to use decapitated specimens, or dorsal sections, in which the viscera were removed and the heart-tube left intact. Such specimens were placed in the fixative for the required length of time, washed and run through the necessary solutions, and embedded in paraffin. When the paraffin had hardened the outer covering was cut away, and with it the hard chitin. This method was very unsatisfactory. The tissue became brittle and dry and chipped away with the chitin, so that complete sections could not be obtained.

I finally hit upon a method that gave favorable results. After exposing the contracting heart to view in its dorsal chitin, a drop of 1 per cent. acetic acid containing methyl green was placed upon the tissue, which at once stopped contracting. As it was desired to examine this preparation under the microscope, I tried to remove a section of it from the chitin and to my surprise found that the whole delicate structure could be entirely separated from the chitin by carefully lifting one end of the soft tissue with the forceps and gently scraping next to the chitinous surface with a scalpel.

I then decapitated an animal and filled the body cavity with the 1 per cent. acetic acid and found that in a short time, three to five minutes, the whole body was so completely loosened from its encasement that, by carefully cutting the chitin along both sides and employing the scalpel, the entire body could be removed, leaving the outer shell quite transparent and entirely free from all of the body-tissue. Even the hard chitinous structures extending into the thorax were loosened from their tissues.

9. McClendon, Biological Bulletin, 1904, VIII, p. 38.

10. Beyne, J., Zeutralblatt für Phys., Bd. XIX, No. 25, 1905, p. 959.

I found that 2 per cent. formic acid would produce the same results, if left on the tissue no longer than five minutes. After having the tissues thus so well removed from the objectionable chitin, they were placed in the several fixatives. It was found, however, that the acetic acid shrank the cells—especially the so-called "ganglion-cells"—and the formic acid did not work well with some of the fixatives.

I next found that the fixatives could be put upon the tissue at once with the same results as the acids used. I cannot say that this is true for all fixatives, but I am inclined to think that it is, since its results were satisfactory for those tried, especially with the chrome-oxalic mixture, the method which I found among the best for this work.

There is one precaution, however, to be observed. The tissue should be removed from the chitin in from three to five minutes after being killed with the fixative. A longer time seems to make the tissue soft and thus impossible of removal without tearing. If left for months in a preservative such as 10 per cent. formalin it gets crumbly or brittle and the results are not satisfactory.

A great many different fixatives and stains were employed, most of which were after the direction of Hardesty,[11] Houser,[12] and Lee,[13] with some few individual variations. Over seventy different preparations were mounted.

Flemmings' fluid, so helpful for cytological purposes, proved to be of no value on the heart-tissue.

Unsatisfactory results were also obtained with picric acid, in which specimens were fixed for two or three days and then washed in alcohol; Gilson's fixed from one to two days; Pereny's fluid for eighteen hours; chrome-silver, as described by Houser; gold chlorid, according to Hardesty (p. 51), and mercuric chlorid and formalin (10 per cent.); silver nitrate, also described by Hardesty (p. 41); and even Bethes' injection method, by Hardesty (p. 43). In the latter case the sections were not well fixed and would not clear up, although the method was carefully tested. Houser reports a similar result with Bethes' method.

Most satisfactory results were obtained with 10 per cent. formalin as a fixative, and iron-hæmatoxylin as a stain; and

11. Hardesty, Neurological Technique.

12. Houser, G. L., Jour. of Comp. Neurology, vol. XI, No. 2, 1901.

13. Lee's Vade Mecum, fifth edition.

the Nissl stain and chrome-oxalic fixative of Graf, as described by Houser. Graf used a mixture composed of 200 cc. of an 8 per cent. aqueous solution of oxalic acid, 150 cc. 95 per cent. alcohol and 150 cc. of aqueous solution of chromic acid. The tissues were fixed about six hours and then washed out with 70 per cent. alcohol, and sections made by the paraffin method.

The Nissl stain (methylen blue 3.75 grams, olive-oil soap 1.75 grams, and 1000 cc. distilled water) was poured steaming-hot over the sections for about five minutes. Differentiation was accomplished with anilin alcohol, and clearing with oil of cajeput.

The erythrosin mixture of Held, also mentioned by Houser, gave good results as a counter-stain.

Sections preserved for several months in 10 per cent. formalin were stained with the Nissl stain with good results.

Van Gehuchten's fixing fluid, according to Hardesty (p. 98), but using Nissl staining fluid and erythrosin for a counter-stain, proved to be good, especially for connective tissue.

The only fixatives that brought out the so-called "ganglion-cells," however, were 10 per cent. formalin and mercuric chlorid or formalin alone, and the chrome-oxalic fixatives. The simple 95 per cent. alcohol fixation, as used by Nissl himself, with the Nissl stain, gave good results, but the chrome-oxalic solution was the more satisfactory.

Sections through the ventral nerve-cord and the thoracic and abdominal ganglia were made with the best methods, in hopes that ganglion- and nerve-cells could be certainly identified and their structures compared with others found in the heart, but satisfactory results were not obtained with any of the many methods used. In fact, structures found in and around the ventral cord did not any more resemble nerve- or ganglion-cells, as we would expect to find them, than did those found in the heart sections. For this reason it has certainly not been proven that nerve- or ganglion-cells do not exist in the grasshopper's heart.

ANATOMY OF THE HEART.

My observations corroborate in most respects the description of the circulatory system in insects, although very little mention is made of the grasshopper. Graber[14] seems to have made a sketch of a cross-section through the heart, but it is

14. Graber, Folsom's Entomology, p. 125.

only diagrammatic. I was not able to obtain his paper. Minot[15] made a histologic study of the grasshopper but did not give much attention to the circulatory system. His paper contains quite a complete bibliography of work done on the insects prior to his publication.

As is described for most insects, I found the pulsating tube to end blindly in the last abdominal segment and the pulsations to extend forward. This was proven by exposing the heart along its whole extent, but leaving it intact in the chitin, and then placing a few drops of Ringer's solution colored with neutral red on the posterior portion. Particles of the red were taken into the heart and carried forward as far as the mesothorax. The contractions did not seem strong enough to force them any further. This experiment confirms the opinion that the blood enters the heart through lateral slits or valves. There is probably a pair of these valves for each segment, although microscopical sections did not reveal any definite valvular structures. A few sections gave evidence of folds in the tube, which were probably the valves.

Figure 1, plate XXXVII, represents the heart in position as it was usually exposed for experimentation. As will be seen, the tube itself shows very little variations in size throughout its whole extent. There is a slight enlargement at each segment, an enlargement in the metathorax, and also one in the mesothorax. In the prothorax it becomes smaller and extends as a delicate tube, quite free from surrounding tissue or pericardial cells, into the head-cavity, where it is lost under a large air-sac just back of the eyes.

No other pulsating tube can be detected in any part of the body, even by the aid of the microscope. It is very probable that this anterior tube, or aorta, empties its fluid into the body-cavity, and by means of the respiratory movements, perhaps, and the pulsating sinuses, both ventral and dorsal, the blood finds its way back to the posterior part of the body, where it again enters through the valves of the heart and is propelled forward. Owing to the extensive system of tracheal tubes and air-sacs, located all over the body, it is probable that the blood never becomes very venous, and the heart's chief function is therefore to keep this fluid in motion and the tissues well bathed with it.

That the heart contraction is not dependent upon extrinsic

15. Minot, C. S., Extracted from the second report of the U. S. Entomological Commission, 1880, p. 196.

influences for its control is shown by several experiments. As was described in my previous paper, the heart will contract many hours after being entirely isolated from all other systems. If all the blood is entirely removed from such an isolated heart (fig. 1) by means of filter-paper, the contractions soon cease. But if Ringer's solution is then placed upon the heart, in the body-cavity, the contractions will be resumed. This fluid seems to act in this respect as the animal's own blood. The heart usually contracts at the same rate throughout its whole extent, but this is not always the case. I have observed different rates in different segments.

An isolated heart may be sectioned at each abdominal segment, and if kept moist with Ringer's solution, each small segment will contract for several hours. In this case the segments do not contract in unison.

HISTOLOGICAL STRUCTURE OF THE HEART AND PERICARDIAL CELLS.

The heart, as seen in cross-section, plate XXXVII, figure 2, lies within the pericardial sinus, and on its dorsal aspect is suspended to the chitin by muscle-fibers and connective tissue. Fibers also lead off from the ventral side of its muscular coat and extend out into the fibrous tissue (*b*, fig. 2), and to the so-called alary muscles. These alary muscles are arranged in fan-shaped bundles, as is shown in figure 1. The wide part extends over the heart, to which it is attached, and the narrow part, or handle of the fan, is attached to the tergite of each segment. Some of the fibers of these muscles extend from their attachment on one side of the wall entirely over to their attachment on the opposite side, while others seem to end in the heart-muscle proper. As viewed from the ventral side, these muscles are seen to contract and expand slightly with the pulsation of the heart, but they also have a pulsation independent of the heart's contraction, and usually at a much slower rate. It is probable that these contractions help the blood to pass through into the pericardial sinus.

The muscle-fibers of the alary system are striated and contain elongated nuclei.

The muscular coat enclosing the heart-cavity is composed of two kinds of cells—one consisting of long muscle-fibers extending around the tube and out into the surrounding tissue as a sort of supporting structure (*f*, fig. 2, and *b*, fig. 7, plate

XXXVII), and the other made up of larger cells as shown in longitudinal section (*f*, fig. 3, and fig. 8). These cells often contain a network of fibers, as seen in figure 8, and also structures resembling nerve-cells (*n*).

In cross-section the heart-muscle appears to be striated, but this is probably due to shrinking of the muscular structure, thus causing the outer tissue-covering to arrange itself in folds.

On either side of the heart-tube is a tracheal tube (figs. 2 and 3, *t*), which makes connection with a spiracle in each segment.

Besides these structures, four distinct kinds of cells are to be found between the alary muscles and the chitin: First, the compact fibrous layer (*b*, fig. 2) next to the alary muscles. Numerous fibers, muscle, and perhaps nerve, extend throughout this layer. The cells are close together, and the nucleus is quite large.

The next and most numerous group of cells is the connective-tissue, or pericardial, cells, which loosely fill the cavity between the more compact ventral and dorsal groups. These cells are shown at *c*, figures 2 and 3, and again at figure 5. Some investigators call them fat-cells, but I see no reason why they should be called that. They are more or less fibrous, too, irregular in shape, vary somewhat in size, and contain a rather large nucleus with many spherical chromatin bodies. Two nuclei are often to be seen in a single cell.

Scattered throughout these cells are structures that very much resemble nerve-cells (*i*, fig. 2), but they are probably only another form of connective-tissue cells.

The most dorsal layer of tissue contains two distinct kinds of cells (fig. 2, *d* and *e*). Those again shown at figure 6 are found in the greatest abundance. They are arranged in rows (usually only one) along the dorsal wall next to the chitin.

The cytoplasm in these cells forms a network throughout the cell. The nucleus is large, with many deeply staining chromatin bodies.

Scattered among these cells, at apparently no regular intervals, are the cells, probably the same as Dogiel called ganglion-cells. These cells are shown at *e*, figure 2, and again a single cell enlarged at figure 4.

These are the cells that are not shown with most of the fixatives. The chrome-oxalic mixture and Nissl stain, or 10

per cent. formalin and iron-hæmatoxylin, show them as illustrated in figure 4. These cells more nearly resemble the ganglion-cells of the vertebrates than any other structures thus far found. They have a distinct, dark-staining nucleolus. The nucleus is quite clear. The cytoplasm is very dense throughout the whole cell.

It is very easy to call these ganglion-cells when one is especially looking for that kind of cells, and yet there is much evidence against their being of this character.

First, in only a few cases have I seen any processes resembling nerve-fibers leading from these cells. Second, they are not confined to any definite place, as one would expect to find ganglion-cells. I have noticed, however, that they are very much more numerous at the place of attachment of the alary muscles to the chitin, and if they are ganglion-cells they may control the action of these muscles as well as those of the heart.

A ventral sinus of similar structure and with similar pulsations has been described as lodging the ventral nerve chain, and these same cells are to be found in this sinus. Ransom gives this as one reason why they are not ganglion-cells, but it seems to me that it is an evidence for, rather than against, such a view. Since these ventral pulsations are similar to the dorsal, it would be expected that they were controlled by the same kind of cells.

Figure 9 is a cross-section through a thoracic ganglion. The methods used did not bring out the nerve- or ganglion-cells very satisfactorily. In fact, very few structures in the least resembling nerve-cells could be seen anywhere excepting around the edges and near the middle of the section. The large round cells scattered around the edge of the section somewhat resemble the large cells seen at figure 6, except that the nucleolus does not stand out so distinctly. In fact, the whole cell does not show a definite structure.

From the results thus far obtained I cannot therefore say that nerve- or ganglion-cells do not exist within the heart-structure of the grasshopper, and I believe they are present and need only the proper stain to prove their existence.